no. 07/787,390, filed November 4, 1991" insert --(abandoned), which is a continuation-in-part of application serial no. 07/432,069 filed November 6, 1989 (abandoned).--

On page 8, line 35, delete "U.S. Patent No. 4,319,216" and insert in its place --U.S. Patent No. 4,399,216 --.

IN THE CLAIMS:

Please amend the claims as follows:

Cancel Claim 1, without prejudice.

26. (amended) A mammalian host cell having a modified endogenous gene comprising [an exogenous] a nucleotide regulatory element different from the wild-type regulatory element normally associated with the endogenous gene integrated, via homologous recombination, into the genome of the mammalian host cell, so that the integrated regulatory element is operatively associated with [an] the endogenous gene of the mammalian host cell and [so that activation and] expression of the endogenous gene [are] is controlled by the [exogenous] integrated regulatory element.

27. (amended) The mammalian host cell of Claim 26 further having an [exogenous] amplifiable gene [operatively associated with the modified gene of Claim 1] integrated into the host cell genome within or proximal to the endogenous

gene, so that the modified endogenous gene is also amplifiable.

28. (amended) The mammalian host cell of Claim 26 or 27 wherein the modified endogenous gene contains at least one mutation introduced [via homologous recombination of the exogenous nucleotide element] into the genome of the mammalian host cell via homologous recombination.

32. (amended) A [The] mammalian host cell [of Claim 27 wherein the exogenous] having an amplifiable gene [is] integrated, via homologous recombination, into the mammalian host cell genome within or proximal to an endogenous gene of the host cell so that the endogenous gene is also amplifiable.

In Claim 33, first line, delete "of Claim 32" and insert in its place -- of Claim 32 or 72 --.

37. (amended) The mammalian host cell of Claim 26, [or] 27, 32 or 72 wherein the mammalian cell is a primary host cell [does not readily grow in culture].

38. (amended) The mammalian host cell of Claim 26, [or] 27, 32, or 72 wherein the mammalian host cell is a primate cell.

In Claim 43, first line, delete "of Claim 27" and insert in it place -- of Claim 27 or 32 --.

44. (amended) A secondary expression host cell having the modified gene of Claim 26, 27, 32 or 72.

Cancel Claim 45, without prejudice.

In Claim 46, second line, delete "or 45".

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48. (amended) A method for producing a mammalian host cell having a modified gene, comprising:

(a) transforming a mammalian host cell with a nucleotide sequence comprising [an exogenous] a nucleotide regulatory element flanked by a nucleotide sequence homologous to a region of [at least about 150 nucleotides of] the host cell genome within or proximal to an endogenous gene present in the mammalian host cell so that the nucleotide regulatory element is integrated via homologous recombination into the genome of the mammalian host cell; and

(b) selecting a transformed mammalian host cell having the modified gene in which the [exogenous] integrated nucleotide regulatory element is operatively associated with the endogenous gene so that [activation and] expression of the endogenous gene [are] is controlled by the [exogenous] integrated regulatory element.

49. (amended) A method for producing a mammalian host cell having an amplifiable modified gene, comprising:

[(c)](a) transforming a mammalian host cell with a nucleotide sequence comprising [an exogenous nucleotide regulatory element,] an [exogenous] amplifiable gene[, and] flanked by a nucleotide sequence homologous to a region of [at least about 150 nucleotides of] the host cell genome within or proximal to an endogenous gene present in the mammalian host cell so that the [exogenous nucleotide regulatory element and the exogenous] amplifiable gene [are] is integrated via homologous recombination into the genome of the mammalian host cell; and

[(d)](b) selecting a transformed mammalian host cell [having the amplifiable modified gene] in which [the exogenous nucleotide regulatory element and the exogenous amplifiable gene are operatively associated with the endogenous gene so that activation and expression of the endogenous gene are controlled by the exogenous regulatory element, and] the amplifiable gene is [operatively associated with] integrated into the host cell genome within or proximal to the endogenous gene [and the exogenous nucleotide regulatory element] so that the endogenous gene [and the exogenous nucleotide regulatory region are] is also amplifiable.

In Claim 50, first line, delete "Claim 48 or 49" and insert in its place -- Claim 48, 75, 49 or 76 --.

51. (Amended) The method of Claim 48, 75 [or] ~~49 or 76~~ wherein the mammalian host cell is a primary host cell [does not grow readily in culture].

In Claim 52, first line, delete "Claim 48 or 49" and insert in its place -- Claim 48, 75, 49 or 76 --.

In Claim 57, first line, delete "Claim 49" and insert in its place -- Claim 75, 49 or 76 --.

In Claim 58, first line, delete "Claim 48 or 49" and insert in its place -- Claim 48, 75, 49 or 76 --.

62. (Amended) A method for producing a secondary expression host cell which expresses [the] a modified gene [of Claim 26], comprising:

[(e)] (a) transforming a secondary expression host cell with nucleic acid encoding the modified gene which was obtained [isolated] from [the] a mammalian host cell [of Claim 26] in which a nucleotide regulatory element was integrated, via homologous recombination, into the genome of the mammalian host cell in operative association with an endogenous gene of the mammalian host cell, so that expression of the endogenous gene is controlled by the integrated regulatory element, and

[(f)](b) selecting a transformed secondary host cell which expresses the modified gene [of Claim 1].

63. (Amended) A method for producing a secondary expression host cell having [the] an amplifiable modified gene [of Claim 27], comprising:

[(g)](a) transforming a secondary expression host cell with nucleic acid encoding the amplifiable modified gene which was obtained [isolated] from [the] a mammalian host cell, [of Claim 27] in which an amplifiable gene was integrated, via homologous recombination, into the host cell genome within or proximal to an endogenous gene so that the resulting modified endogenous gene is also amplifiable; and

[(h)](b) selecting a transformed secondary expression host cell which contains the integrated amplifiable gene [of Claim 27] and expresses the amplifiable modified gene [of Claim 27].

In Claim 64, first line, delete "Claim 62 or 63" and insert in its place -- Claim 62, 82, 63 or 83 --.

In Claim 65, first line, delete "Claim 62 or 63" and insert in its place -- Claim 62, 82, 63 or 83 --.

67. (amended) A method for activating an endogenous gene in a mammalian host cell, comprising:

[(i)] (a) transforming [a] the mammalian host cell with a nucleotide sequence comprising [an exogenous] a nucleotide regulatory element flanked by a nucleotide sequence homologous to a region of [at least about 150 nucleotides of]

the host cell genome within or proximal to an endogenous gene present in the mammalian host cell so that the nucleotide regulatory element is integrated via homologous recombination into the genome of the mammalian cell; and

[(j)] (b) selecting a transformed mammalian host cell in which the [exogenous] integrated nucleotide regulatory element is operatively associated with the endogenous gene so that expression of the endogenous gene is activated by the exogenous regulatory element [and the endogenous gene expresses its gene product].

69. (amended) A method for producing a recombinant protein, comprising culturing a mammalian host cell having a modified gene comprising [an exogenous] a nucleotide regulatory element [operatively associated] integrated, via homologous recombination, into the genome of the host cell [,] in operative association with an endogenous gene of the mammalian host cell so that [activation and] expression of the endogenous gene [are] is controlled by the [exogenous] integrated regulatory element, under conditions wherein the modified gene is [activated] expressed and the recombinant protein encoded by the modified gene is produced.

70. (amended) The method of Claim 69 or 91 further comprising isolating the recombinant protein produced by culturing the mammalian host cell.

Cancel Claim 71, without prejudice.

Insert new Claims 72-104 as follows:

After Claim 32, insert new Claim 72:

-- 72. (new) The mammalian host cell of Claim 32 further having a nucleotide regulatory element different from the wild-type regulatory element normally associated with the endogenous gene integrated, via homologous recombination, into the genome of the mammalian host cell, so that the integrated regulatory element is operatively associated with the endogenous gene and controls expression of the endogenous gene. --.

After Claim 43, insert new Claims 73-74:

-- 73. (new) The mammalian host cell of Claim 26 or 72 wherein the regulatory element is a promoter, promoter/enhancer, or enhancer.

74. (new) The mammalian host cell of Claim 26 or 72 wherein the regulatory element is the enhancer and promoter of the immediate early gene of human cytomegalovirus. --

After Claim 48, insert new Claim 75:

-- 75. (new) The method of Claim 48 wherein the nucleotide sequence used to transform the mammalian host cell further contains an amplifiable gene which is integrated, via homologous recombination, into the host cell genome within or proximal to the endogenous gene so that the endogenous gene is also amplifiable. --.

After Claim 49, insert new Claims 76-78:

-- 76. (new) The method of Claim 49 wherein the nucleotide sequence used to transform the mammalian host cell further contains a regulatory element which is integrated, via homologous recombination, into the host cell genome so that the regulatory element is operatively associated with the endogenous gene and controls expression of the endogenous gene.

77. (new) The method of Claim 48 or 75 wherein the regulatory element is a promoter, promoter/enhancer, or enhancer.

78. (new) The method of Claim 48 or 75 wherein the regulatory element is the enhancer and promoter of the immediate early gene of human cytomegalovirus --.

After Claim 50, insert new Claims 79-81:

-- 79. The method of Claim 50 wherein the selectable marker is the neo gene selected with G418, the herpes virus tk gene selected with HAT medium, or the gpt gene selected with mycophenolic acid.

80. (new) The method of Claim 48, 75, ~~49 or 76~~ or 49 wherein the nucleotide sequence used to transform the mammalian host cell further comprises a negative selectable marker, and selecting a transformed host cell that did not integrate the negative selectable marker.

81. (new) The method of Claim 80 wherein the negative selectable marker is the herpes virus tk gene negatively selected with gangcyclovir or acyclovir --.

After Claim 62, insert new Claim 82:

-- 82. (new) The method of Claim 62 in which the mammalian host cell further contains an amplifiable gene integrated, via homologous recombination, into the genome of the mammalian host cell within or proximal to an endogenous gene of the mammalian host cell so that the endogenous gene is also amplifiable. --.

After Claim 63, insert new Claims 83-85:

-- 83. (new) The method of Claim 63 in which the mammalian host cell further contains a regulatory element integrated, via homologous recombination, into the genome of the mammalian host cell in operative association with an endogenous gene, so that expression of the endogenous gene is controlled by the integrated regulatory element.

84. (new) The method of Claim 62 or ~~83~~ 82 wherein the regulatory element is a promoter, promoter/enhancer, or enhancer.

85. (new) The method of Claim 62 or ~~83~~ 82 wherein the regulatory element is the enhancer and promoter of the immediate early gene of human cytomegalovirus. --.

After Claim 64, insert new Claims 86-88:

-- 86. (new) The method of Claim 64 wherein the selectable marker is the neo gene selected with G418, the herpes virus tk gene selected with HAT medium, or the gpt gene selected with mycophenolic acid.

87. (new) The method of Claim 62, 82, ~~63 or 83~~ 0r63 wherein the nucleic acid used to transform the secondary expression host cell further comprises a negative selectable marker, and a transformed host cell that did not integrate the negative selectable marker is selected.

88. (new) The method of Claim 87 wherein the negative selectable marker is the herpes virus tk gene negatively selected with gangcyclovir or acyclovir --.

After Claim 67, insert new Claims 89-90:

-- 89. (new) The method of Claim 67 wherein the regulatory element is a promoter, promoter/enhancer, or enhancer.

90. (new) The method of Claim 67 wherein the regulatory element is the enhancer and promoter of the immediate early gene of human cytomegalovirus. --.

After Claim 69, insert new Claim 91:

-- 91. (new) The method of Claim 69 in which the mammalian host cell further has an amplifiable gene integrated, via homologous recombination, into the host cell genome within or proximal to the endogenous gene so that the resulting modified gene is also amplifiable, and the culturing is performed under conditions wherein the modified gene is amplified --.

After Claim 70, insert new Claims 92-104:

-- 92. (new) A method for producing a recombinant protein, comprising culturing a mammalian host cell in which an amplifiable gene was integrated, via homologous recombination, into the genome of the host cell within or proximal to an endogenous gene of the host cell, so that the endogenous gene is also amplifiable, under conditions which amplify both the amplifiable gene and the endogenous gene, so that expression of the recombinant protein encoded by the endogenous gene is enhanced.

93. (new) The method of Claim 92 in which the mammalian host cell further has a nucleotide regulatory element integrated, via homologous recombination, into the host cell genome in operative association with the endogenous gene, so that expression of the endogenous gene is controlled by the integrated regulatory element, and the culturing is performed under conditions wherein the endogenous gene is expressed.

94. (new) The method of Claim ~~92 or 93~~ 69, 91 or 93 further comprising isolating the recombinant protein produced by culturing the mammalian host cell.

95. (new) The method of Claim 69 or 91 wherein the regulatory ~~element~~ sequence is a promoter, promoter/enhancer, or enhancer.

96. (new) The method of Claim 69 or 93 wherein the regulatory ~~element~~ is the enhancer and promoter of the immediate early gene of human cytomegalovirus.

97. (new) A method for producing a recombinant protein, comprising culturing a secondary expression host cell having a modified gene obtained from a mammalian host cell in which a nucleotide regulatory element was integrated, via homologous recombination, into the genome of the mammalian host cell in operative association with an endogenous gene of the mammalian host cell so that expression of the endogenous gene is controlled by the integrated regulatory element, under conditions wherein the modified gene is expressed and the recombinant protein encoded by the modified gene is produced.

98. (new) The method of Claim 97 in which the secondary expression host cell further has an integrated amplifiable gene operatively associated with the modified gene, and the culturing is performed under conditions wherein the modified gene is amplified and expression of the recombinant protein is enhanced.

99. (new) The method of Claim 97 or 98 further comprising isolating the recombinant protein produced by culturing the secondary expression host cell.

100. (new) A method for producing a recombinant protein, comprising culturing a secondary expression host cell having a modified gene derived from a mammalian host cell in which an amplifiable gene was integrated, via homologous recombination, into the genome of the mammalian host cell within or proximal to an endogenous gene of the mammalian host cell so that the resulting modified gene is also amplifiable, under conditions wherein the modified gene is amplified and expression of the recombinant protein is enhanced.

101. (new) The method of Claim 100 wherein the secondary host cell further has an integrated regulatory element operatively associated with the modified gene, and the culturing is performed under conditions wherein the modified gene is expressed.

102. (new) The method of Claim ~~100 or 101~~ 97, 98, or 101 further comprising isolating the recombinant protein produced by culturing the secondary expression host cell.

103. (new) The method of Claim 97 or ~~101~~ 98 wherein the integrated regulatory element is a promoter, promoter/enhancer, or enhancer.

104. (new) The method of Claim 97 or ~~101~~ 98 wherein the integrated regulatory element is the enhancer and promoter of the immediate early gene of human cytomegalovirus. --.